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Concentration–Time Relationship for Various Regimens of Inhalation of Organic Compounds

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An attempt was made to establish a concentration–time relationship for continuous and interrupted inhalation of a number of nonelectrolytes to establish norms for the permissible content of harmful substances in atmospheric air. The tests showed that the indicated relationship can be expressed in the form of a straight line on a logarithmic grid by using either acute toxicity parameters or the onset of physiological and biochemical shifts. This makes it possible to predict the chronic effect thresholds of substances on the basis of the results of short-term experiments.

In modern conditions of vigorous development of industry, transportation, and agriculture, rapid determination of the permissible level of harmful substances in the ambient air and assessment of the health impact of intermittent exposure to them are necessary if timely action is to be taken to prevent chemical pollution of the environment.

The development of methods for ascertaining the "isoeffectiveness" of concentrations of a substance in the air under different modes of its ingestion into the body can assist in the search for answers to these questions.

Determination of biological equivalence (isoeffectiveness) of concentrations in the environment is vital in the general qualitative evaluation of the interaction between harmful substances and the organism; it is therefore necessary to decide which quantitative relationship (dose–effect, time–effect, and dose–time) should be used for these purposes.

In most research in the field of toxicology today, toximetric parameters are determined from study of the dose (concentration)–effect relationship: the parameters of acute toxicity are defined in terms of high concentrations (average lethal concentrations LD_{50}, threshold of acute effect), while the parameters of chronic effect are defined in terms of low concentration levels (threshold of chronic effect and no-effect dose). Indices of degree of hazard of a substance, such as the area of acute and chronic effect and the coefficient of accumulation, are determined at the same time.

A precisely fixed time span is crucial in determining toximetric indicators from study of the dose (concentration)–effect relationship (*1–6*).

In this regard, use of the concentration–time relationship, under which the toxic (for example, threshold) effect remains constant while time and concentration vary, can contribute greatly to a prognosis of the biological equivalency of concentrations of a substance in the environment.

Since the quantitative expression of the concentration–time relationship has been inadequately studied, we sought in the present research to clarify its character by the parameters of acute toxicity and certain physiological and biochemical indices under different conditions of exposure of animal organisms to a number of organic compounds.

Under our experimental plan, in order to ascertain the possibility of expressing quantitatively the concentration–time relationship under conditions of continuous inhalation of volatile substances, we subjected the animals to not less than four or five

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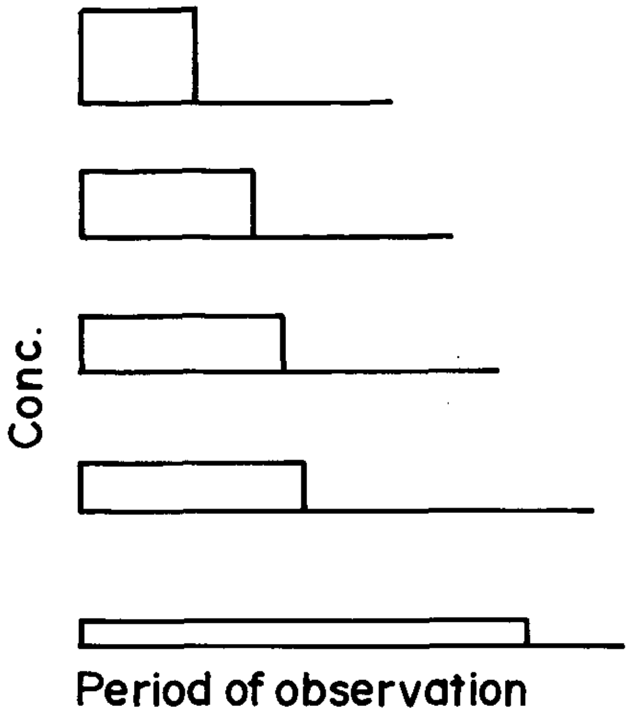

FIGURE 1. Schematic diagram of continuous inhalation action of chemical compounds at various concentrations

concentrations, ranging from relatively high, through medium, to low (Fig. 1).

In our experimental investigation of the concentration–time relationship under conditions of intermittent inhalation of volatile substances, we subjected the laboratory animals to a single concentration in three to five different modes (Fig. 2).

Repeated exposure for equal time periods alternated with intervals in which the substance was completely lacking in the test atmosphere. For any given group of animals, the length of the intervals between the repeated exposures was held constant throughout the experiment, but it was different for different groups. This means that the length of a single exposure-plus-interval cycle was different for each group. From a knowledge of the length of a single exposure and the length of a cycle under a given regimen, one can easily determine the value of the average concentration of the substance by its concentration during the exposure period.

The concentration–time relationship was studied with various classes of nonelectrolytes such as aliphatic hydrocarbons (gasoline), chlorinated hydrocarbons (carbon tetrachloride, dichloroethane), alcohols (methyl, ethyl, amyl), ketones (acetone), aldehydes (acrolein), aromatic compounds (benzene, xylene, toluene, styrene), and their amino derivatives (aniline), the toxicological properties of which are well known.

Research on the influence of these substances administered to animals in different concentrations and regimens was conducted on two species of animals: white mice and white rats. The concentration–time relationship was studied on the mice by the parameters of acute toxicity and on the rats by using physiological and biochemical indices. A smaller number of substances, including benzene and aniline, were used in the latter case. Since these substances have a marked effect on the central nervous system and circulatory system of animals, we studied the chronaxy ratio of antagonist muscles, the whole blood cholinesterase activity, and the amount of erythrocytes, leukocytes, erythrocytes with Heinz bodies, reticulocytes, and methemoglobin in the blood.

In studying the continuous and interrupted action of harmful substances, we sought to determine the onset time of one or another toxic effect. The average time to loss of righting reflex and death of the animals (average effective time ET_{50}) was computed by the method of Litchfield and Wilcoxon (*1*) as modified by Prozorovskii (7).

The time of occurrence of individual physiological and biochemical changes in the animals was determined by studying the dynamics of these indicators under the impact of exposure to harmful substances. In doing this, we tried to ensure that the number of repeated measurements during the process of action of a substance at a given concentration or regimen was in no case less than 5–8. We accepted the time at which the first statistically reliable change in the relevant indicator occurred as the time of onset of individual physiological and biochemical changes.

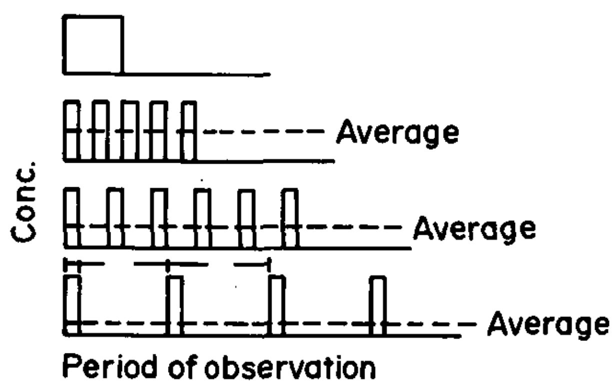

FIGURE 2. Schematic diagram of regimens for studying the effect of inhalation of chemical compounds on the animal organisms.

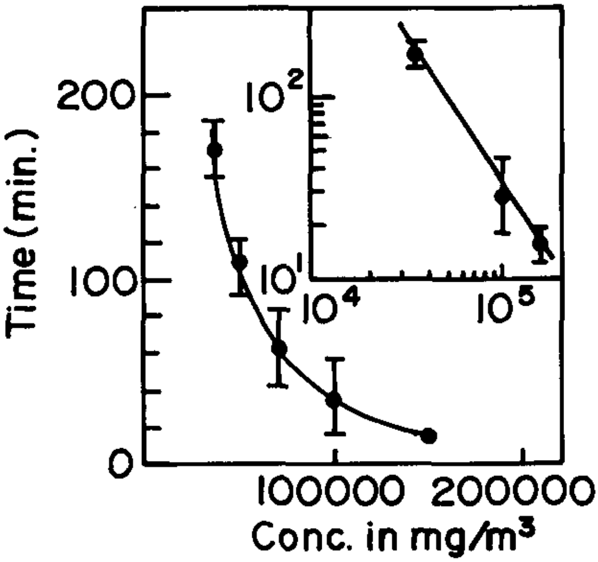

FIGURE 3. Dependence of the time of death of 50% of the mice *on benzene concentration in the air with continuous in-*halation on a normal scale and (inset) logarithmic scale.

Graphic analysis of the data from investigation of the impact of continuous inhalation of organic compounds of different types revealed that the concentration–time relationship as reflected in the parameters of acute toxicity (lateral position and death of 50% of the animals) can be expressed as a straight line on a logarithmic plot (Fig. 3). In this connection, the angle of inclination of concentration–time curves varies within rather broad limits (from 110° inhalation of dichloroethane to 140° for the inhalation of acetone).

Similarly, this relationship can be portrayed by physiological and biochemical changes for continuous inhalation of benzene and aniline by white rats (Fig. 4).

Since the concentration–time relationship can be shown to be linear on a logarithmic grid, it is possible, in accordance with the equation for the line $y = ax + b$, to express this relationship in the formula $\log t = \log t_0 - \tan \alpha (\log C)$, where t is the time of onset of a given toxic effect when the substance is inhaled continuously at concentration C; t_0 is the time of manifestation of the same effect with concentration C_0 and taken as the unit of time measurement (minutes, hours, days, etc.); and α is the angle of inclination of the straight line to the abscissa (concentration).

The formula obtained by us supports the conclusion of Lazarev and Brusilovskaya (*8*) and also of Slavgorodskii (*9*) that Haber's well-known formula does not always correctly reflect the relationship between exposure and concentration. According to the expression derived by us, however, Haber's formula expresses that relationship in the particular case when $\tan \alpha = 1$. The relationship of the time of onset of carboxyhemoglobinemia in humans to the carbon monoxide content of the air (*10*) and even *the dependence of the onset time of narcosis in frog* muscles on the concentration of many substances in Ringer solution (*11*) conform with the same expression.

In studying the effect of periodic inhalation *under the plan we used, we were able to compare* the effect, on the one hand, of interrupted inhalation, where the magnitude of the maximum concentrations equalled that of the continuously inhaled concentrations and, on the other, of intermit*tent exposure, with the equality of concentrations* established by their average magnitudes.

As our criterion for the effect of interrupted and intermittent exposure, we chose the overall time of onset of the given toxic effect (total time of all the repeated exposures plus the intervals between exposures).

Graphic analysis of the research results revealed that interrupted exposure, judging from the parameters of acute toxicity (lateral position and death of 50% of the mice), is in most cases less dangerous than continuous exposure to the substance, since the overall time of onset of these effects increases. As the intervals between repeated inhalations grow longer, the toxic effect of some substances (acetone and amyl, ethyl, and methyl alcohol) declines proportionately, (Fig. 5) while that of other substances (benzene, toluene, xylene, styrene) declines almost exponentially, but to different degrees. For such substances as carbon

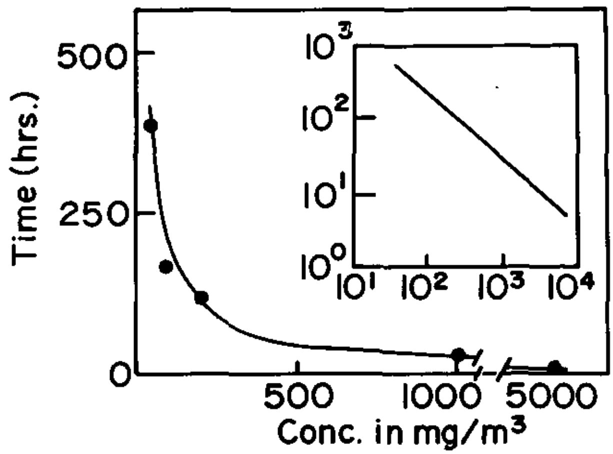

FIGURE 4. Dependence of the time of onset of inverse antagonist muscle chronaxy relationship in white rats on benzene concentration in the air with continuous inhalation on a normal scale and (inset) logarithmic scale.

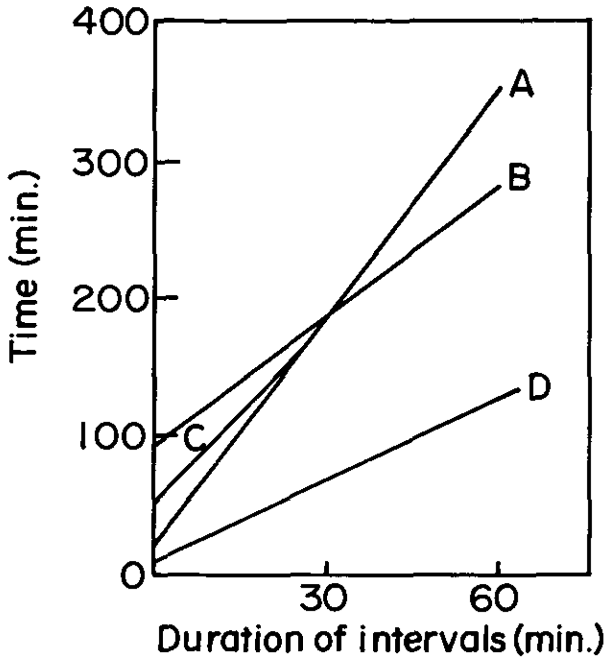

FIGURE 5. Dependence of the total time to onset of the loss of righting reflex for 50% of the mice on duration of intervals between the repeated effects of (*A*) amyl alcohol, (*B*) methyl alcohol, (*C*) ethyl alcohol, (*D*) acetone.

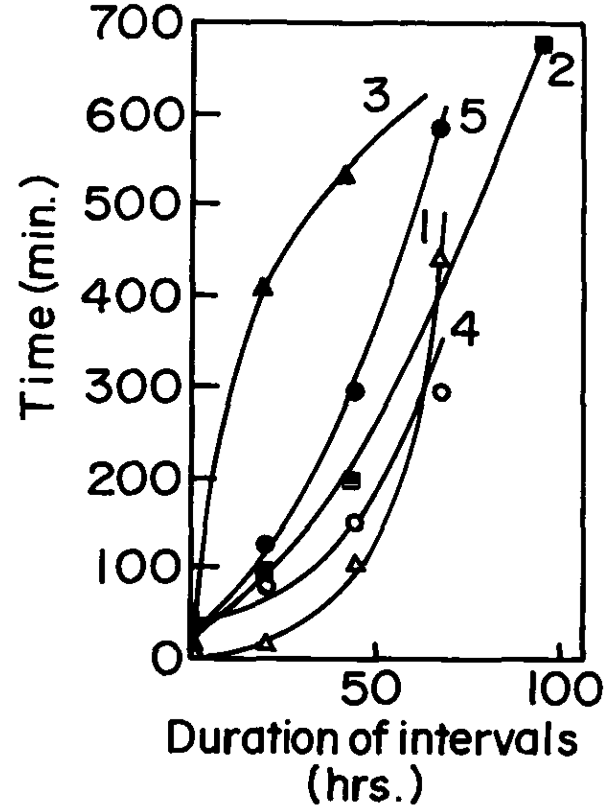

FIGURE 6. Total time for the manifestation of (*1*) methemoglobinemia, (*2*) erythrocytes with Heinz bodies, (*3*) reticulocytosis, (*4*) lowering of blood cholinesterase activity, and (*5*) inverse chronaxy relationship in white rats as a function of the duration of the intervals between the repeated 4-hr effects of aniline at a concentration of 18 mg/m^3.

tetrachloride and acrolein, the curves for the total time, depending on the length of the intervals between exposures, were irregular. Moreover, the time of onset of toxic effects was actually somewhat reduced in the case of carbon tetrachloride and gasoline when the intervals were short, indicating the possibility that the adverse effect of these substances is greater than when exposure to them is continuous.

As indicated by physiological and biochemical changes in white rats, interrupted exposure to benzene and aniline has a less pronounced impact on the functional state of the central nervous system and blood than does continuous exposure (Fig. 6).

At the same time, similar regimens of exposure to a substance in a given concentration can alter to different extents the overall time of onset of given changes in the organism. In some cases, the overall time of onset first increases slowly and then quickly when the length of the intervals is increased. In other cases the reverse occurs: the time first increases rapidly and then slowly. The nonuniform nature of the reactions of the various systems of the organism to the discontinuous exposure is obviously related to the complexity associated with the processes of adaptation (compensatory processes) and the accumulation effect in these systems.

Using the average concentrations of a substance in conditions of periodic exposure and the overall time of onset of given toxic effects, one can construct curves for the concentration–time relationship which, considered together with the analogous curve reflecting the onset of the same effects under conditions of continuous inhalation, permit an assessment of the substance's intermittent effect.

Assessment of intermittent exposure by the parameters of acute toxicity reveals that substances which are easily soluble in water (acetone, methyl and ehtyl alcohol,) do not intensify the toxic effect, and in some concentrations and regimens even lead to a weakening of the toxic effect.

On the other hand, substances with relatively poor solubility in water (dichloroethane, carbon tetrachloride) can lead to an intensification of the toxic effect when the intervals are short and the exposure is intermittent. This is in agreement with the data of Tolokontsev (*12*), Lyublina and Mikheev (*13*), and Ulanova et al. (*14*).

Evaluation of intermittent exposure to benzene and aniline by the changes that occur in physiological and biochemical indicators reflects the complexity of the manifestation of the body's responses to such exposure. Different systems react differently to the same intermittent exposure, and when one system shows a weakened effect, another shows a stronger effect, and a third shows neither.

The extreme complexity of the body's responses to the substances in different regimens seems to be correlated with changes in the condition of the body's compensatory processes and in the accumulation of the toxic agent. These subjects must be pursued in special research.

On the basis of the present study, the ability to express graphically the dependence of the time of onset of given toxic effects for different regimens of exposure can serve as the basis for predicting the biological equivalence of concentrations of a substance inhaled continuously or intermittently, if equal time of onset of the effects is accepted as the criterion of equivalency.

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